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Characterization and Optimization of Factors Affecting Growth and Pigment Production of Bacterium Isolate Mif41

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Abstract
At present, due to the health side effects of chemically synthesized coloring agents, naturally produced coloring agents were favored. From all naturally produced coloring sources, microbial pigments have been given precedence, due to easy manipulation of the microorganisms and optimization of the production processes. The main aim of this article is to report the characteristics and factors affecting growth and pigment production by bacterium isolate Mif41. The soil samples were collected and serially diluted from 10^{-1} to 10^{-6} and each dilution was plate on the sterilized Glucose, mannitol, Tryptose Yeast extract Agar (GMTYEA) medium and incubated at 28°C under aerobic condition. After 48h of incubation the yellow pigmented bacterium isolate was picked and purified by repeated streaking on GMTYEA and the pure culture was maintained on slant at 4°C. The pure culture of the bacterium isolate was characterized morphologically and biochemically. Both extracellular and intracellular pigments obtained from the bacterium isolate were analyzed by using UV-visible spectrophotometer. Effect of carbon sources, nitrogen sources, medium PH, incubation temperature and effect of salt concentrations on growth and pigment production were evaluated. The morphological and biochemical characterization of the bacterium isolate tentatively suggested as Pseudomonas sp. From the different carbon sources tested fructose favored maximum extracellular and intra cellular pigmentation while sorbitol was found minimal in this respect. All the organic sources of nitrogen stimulated growth and pigmentation while all the inorganic sources of nitrogen were inhibitory for growth and pigmentation. Incubation temperatures of 20-28°C were favored growth and pigmentation while incubation temperature above 30 were highly limiting for growth and pigmentation. Medium PH 8 found to be favorable for growth and pigmentation while acidic and basic pH were limiting for growth and pigmentation. Lower salt concentration was found to favor the growth and pigmentation, while increased concentration were found to be limiting. The results of this study clearly indicated that the bacterium isolate Mif41 is able to produce different colored pigments under different nutritional and environmental conditions suggesting that this bacterium isolate will be considered as a candidate for the industrial production of pigments which will be applied in various industries.
Key words/phrases: Bacterium isolate, Natural color, pigment, Rhizosphere soil, Synthetic color
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Introduction

Pigments are the chemical substances that absorb the light of visible region and with uniqueness of significance to many industries (Desai, 2018). They produce the colors that are sensible at each pace of our lives and are in leaves, fruits, vegetables and flowers. They are also present in skin, eyes, and other animal structures and in bacteria and fungi.

Pigments are used for coloring of food; cloth, painting, cosmetics, pharmaceuticals and plastics (Dufosse, 2009) and they provide attractive appearance to those products (Kumar *et al.,* 2015). Earlier, chemically synthesized colors dominate the market, since they were easier to produce, less expensive, and superior in coloring properties. However, in recent time consumer pressure, sociological changes and technological advances leading to more proceeds in food processing industry have increased the overall color market (Downm and Collins, 2000). Synthetic dyes generally contain benzene backbone moiety (aromatic) that makes them more recalcitrant in nature. Their toxicity is increased by substituent like nitro, methyl, methoxy or halogen groups. Food and drug administration reported that dyes containing metals such as lead, chromate and copper sulphate possess the potential risk of causing serious health and environmental hazards (FDA, 1993).

Natural colorants or dyes derived from flora and fauna are believed to be safe because of non-toxic, noncarcinogenic and biodegradable in nature. The advantages of pigment production from microorganisms encompass easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades. Pigments of various colors are synthesized to protect the cells of microorganisms from injurious effect of light rays of visible and near ultraviolet range (Sinha *et al*., 2017). Bacteria produce pigments for various reasons and it plays an important role. Pigment production in bacteria is associated with morphological characteristics, cellular activities, pathogenesis, protection and survival. Pigments come in a wide variety of colors, some of which are water soluble (Tibor, 2007). Pigments obtained from microorganisms have numerous biological activities like antioxidant, anticancer, immunosuppressive, antimicrobial (Abhishek *et al.,* 2015).

These pigments are synthesized by various types of microorganisms as secondary metabolites and not often

found in all types of organisms. Micro-organisms which have the ability to produce pigments in high yields include species of *Monascus*, *Paecilomyces*, *Serratia*, *Cordyceps, Streptomyces* and yellow-red and blue compounds produced by *Penicillium herquei* and *Penicillium atrovenetum*, *Rhodotorul*a, *Sarcina*, *Cryptococcus*, *Monascus purpureus*, *Phaffia rhodozyma*, *Bacillus* sp., *Achromobacter*, *Yarrowia* (Joshi *et al*., 2003). Microorganisms produce various pigments like carotenoids, melanins, quinones, flavins, prodigiosin sand more specifically monascins, violacein or indigo (Keneni and Gupta 2011; Sasidharan *et al*., 2013; Tarangini et *al*., 2013; Moss 2002).

The utilization of natural pigments in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes has been mounting (Tibor 2007). In the food industry they are used as additives, antioxidants, color intensifiers, etc. Microbial colorants play a significant role as food coloring agent, because of its production and easy downstream processing (Malik *et al*., 2012, Venil *et al*., 2013).

Most of the chemically based food colorants are derived from petrochemicals. The bio-safety of long term ingestion of these substances has started evoking a considerable consumer concern over and increasingly stringent restrictions in near future is to eliminate some of the currently approved synthetic colorants. For example, fast green dye, widely used as food colorant, has been shown to be an immunotoxic agent (Golka *et al*. 2004). Due to eventual harmful effects of synthetic pigments and marketing advantages of employing natural ingredients to consumer concerns, the utilization and demand of natural pigments in different industries has been increasing (Dufosse, 2009).

Thus, microbial species represent a new source of pigments having applications that are more diverse compared to plant pigments. Just like other microbial products, microbial pigments production holds specific advantages of higher growth rate in fermentative production and further increase in geometric proportions through genetic engineering over synthesis of artificial and inorganic colors through chemical processes. As a result, the search for new microbial species/ strain capable of producing new pigment(s) with diverse use has come up as a potentials research direction. Over all investigation involving, isolation, characterization of pigment producing microorganisms and application of the pigment produced by the microorganisms has not yet given much attention in Ethiopia. So, this article reports the characteristics and factors affecting growth and pigment production from yellow pigmented bacterium isolated from tree rhizophere.

Materials and methods

Soil samples collection for isolation of pigment producing bacteria.

Soil samples were collected from the rhizosphere of eight (8) different trees of which four them were endemic and four of them were exotic. These were: *Cordia sp*, *.Acacia* sp,*Syzgiumguineense*, *Millettia sp*, *Juniprus*, *Gravillae*, *Eucalyptus* and *Jathropha*. One kilogram of rhizosphere soil samples were collected at depth of 30cm from the surfaces of the roots, in a polyethylene bag and were immediately transported to the microbiology laboratory department of biology, College of natural and computational sciences Ambo University.

Preparation serial dilution of soils for pigmented bacterial isolates

One gram of soil was put in to 9ml of distilled sterilized water which was then form 10^{-1} dilution and one ml of 10^{-1} dilution was transferred in to a test tube containing 9ml distilled sterilized water so as to from 10^{-2} dilution. This procedure was continued until 10^{-6} dilution was formed. Then one ml of the three last dilutions were pour plated on the Petri-dishes containing Glucose, Mannitol, Tryptone Yeast extract Agar medium (GMTYEA) for the isolation of the pigmented bacteria and the inoculated plates were incubated at 28^0C under aerobic condition. The composition of GMTYEA g/l was: Glucose 5, mannitol 4, Tryptone 10, Yeast extract 3, NaCl 5, $MgSO_2$. $7H_2O$, agar 20 (Keneni and Gupta 2011). Each component of the medium was weighed on sensitive electrical balance and dissolved in 1000ml distilled sterilized water and boiled on hot plate to dissolve the components uniformly and sterilized in the autoclave at 15psi, 121°C, for 30 minutes. After sterilization the medium was poured into Petri dishes and kept in laminar follow hood to cool. Then 0.1ml of appropriate serial dilution of the soils were spread plated in triplicate and inoculated palates were incubated in the incubator at 28°C for 48 hrs. Although a number of pigmented bacteria were isolated from the rhizosphere of the different trees only the deep yellow pigmented bacterium isolated from the rhizosphere soils of *Millettia sp*, *was* selected for further characterization and optimization of conditions for maximum growth and pigmentation.

Morphological and Biochemical characterization of the yellow pigmented bacterium isolate Mif41

Colony characterization of yellow pigment producing bacteria from GMTYEA plate (48 hour incubation)was done based on its size, shape, color, margin, opacity, consistency, elevation, Gram staining and motility.

The biochemical tests performed were Indole test, Methyl Red (MR), Voges Proskauer (VP), Simmon's Citrate test, Oxidase test, Catalase tests, urea hydrolysis tests, H_2S production testes which were recommended in the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994, Ewing 1986).

Extractions of pigments from yellow pigmented bacterium isolate Mif41

The yellow pigmented bacterium isolate was observed to produce different colored pigments both in the extracellular and in the intracellular under various nutritional and physiological conditions. The extracellular pigments were collected by centrifuging a broth culture at 10,000 rpm for 5min, collecting the supernatant pigments and the pellets were re-suspended in acidified methanol (1 : 50 v/v) HCL and methanol. Then the mixture was vortexed and the suspension was centrifuged at 10,000rpm for 5 min and supernatant was collected. Suspending in the solvent mixture and centrifugation was repeated till the pellet changes to colorless. After centrifugation, supernatants containing diffused pigments were filtered through Millipore membrane filter (pore diameter 0.22µm). The absorbance of both the extracellular and intracellular pigments were measured on UV-visible spectrophotometer in the range 200-800nm (Sinha *et al*. 2017).

Effect of nutritional and physical factors affecting growth and pigmentation

Carbon sources on growth and pigmentation of bacterium isolate Mif41

The effect of different carbon sources on the growth of the bacterium isolate was studied by supplying different carbohydrates as sole sources of carbon and energy (at 0.1 per cent level) in GMTYE basal medium (less glucose and mannitol). These carbohydrates included different monosaccharides (Fructose, glucose, mannitol, Myo-insitols and sorbitol), disaccharides (lactose, maltose, sucrose) and polysaccharides (starch). For this purpose, GMTYE basal medium was prepared in one-liter capacity Erlenmeyer flask and 50 ml aliquot of the medium in separate 250 ml Erlenmeyer flask was amended with 0.05 g of the specific carbon source to be studied and the media were sterilized at 15psi, 121°C, for 30 minute in autoclave. After sterilization the medium were cooled and inoculated with 0.1 ml of bacterial suspension and incubated at 28°C. After 48hrs incubation period, the growth and pigmentation of the bacterium isolate was evaluated (Shyam and Saramma, 2017).

Nitrogen sources on growth and pigmentation of bacterium isolate Mif41

The effect of different organic and inorganic nitrogen sources on the growth and pigmentation of the bacterium isolate was tested by supplying different nitrogen sources as sole sources of nitrogen in GMTYE basal medium (less yeast extract and tryptone). The different nitrogen sources studied included organic (beef extract, peptone, tryptone, yeast extract, milk powder) and inorganic (KNO_3, urea and $(NH_4)_2SO_4$). The basal medium of GMTYE was prepared in one liter capacity Erlenmeyer flask and 50 ml aliquot of the medium 250ml Erlenmeyer flask was separately amended with the a specific nitrogen sources (at one per cent level). The prepared media was sterilized at 15psi, 121°C, for 30 minute minutes. The cooled medium was inoculated with 0.1ml of bacterial culture and incubated at 28°C,for 48hrs, the growth and pigmentation of the bacterium isolate in the medium was evaluated (Cecilia *et al.,* 2020).

Effect of pH on growth and pigmentation of bacterium isolate Mif41

In order to determine the effect of initial pH of the medium on growth of the bacterium isolate, GMTYE medium was prepared with different pH, such as 4.5, 6.0, 7.0, 8.0 and 10.0. For this purpose, GMTYE medium was distributed in 50ml aliquot in to 250 ml capacity Erlenmeyer flask and the pH of each medium was adjusted to required value (by using 1N NaOH and 1N HCl). Thereafter, all media was sterilized at 121°C using 15psi of steam for 30 minutes. Liquid medium was inoculated with 0.1 ml test bacterial isolate and incubated at 28°C, for 48hrs incubation period, the growth of the bacterial isolate was observed visually for relative growth, cell mass collected by centrifugation at 100 for 2minrpm. The pigment from cell pellet was extracted with organic solvent (Shivalkar and Prabha, 2014).

Effect of different incubation temperature on growth and pigmentation of bacterium isolate Mif41

Effect of different incubation temperature (20°C, 25°C, 28°C, 32°C and 37°C and 45°C) on the bacterial isolate cell growth and pigment production was tested on complete GMTYE broth medium. The GMTYE broth medium was prepared and 50 ml aliquot was distributed into 250ml capacity Erlenmeyer flask. The medium was sterilized at 121°C using 15psi of steam for 30 minutes. After sterilization, the medium was cooled to room temperature and inoculated with 0.1ml suspension of the bacterial isolate and incubated at 28°C. After 48hr incubation period, the growth of the bacterial isolate was observed visually for relative growth and pigmentation (Shoumita and Shweta, 2018).

Effect of different concentration of salt (NaCl) on growth and pigmentation of bacterium isolate Mif41

Effect of different concentration of salt (sodium chloride) on growth and pigment production by the bacterial isolate was tested by growing in different concentration of sodium chloride (0.5, 1.0, 2.0, 3.0, 4.0, 5.0%) in GMTYE basal medium (less NaCl) was conducted as follows. The GMTYE basal medium was prepared and distributed in 50ml aliquots into 250 ml capacity Erlenmeyer flask followed by addition of NaCl at different levels. The medium was sterilized at 121°C using 15psi of steam for 30 minutes, the medium was cooled to room

temperature and inoculated with 0.1 ml suspension of the bacterial isolate and incubated at 28°C for48hours. (Shivalkar and Prabha, 2014).

Data analysis

Quantitative data were analyzed using Statistical Package for Social Sciences (SPSS) version 21. To compare the level of Absorbance (ABS) of different pigments, one way analysis of variance (ANOVA). Statistical test was set at 95% confidence level.

Results and Discussion

Isolation of Pigment Producing Bacteria

From the different soils samples collected from the rhizosphere of some tree from the main campus of the Ambo University, a number of colored colonies of bacteria were isolated, for further characterization, growth and pigment production optimization studies, the extensively yellow pigmented isolate Mif41 was selected.(Fig 1). The results of this study is in line with the reports in the literature, that stated that, yellow pigmented bacteria are widely present in soil and based on the isolates found they can effectively produce amylase enzyme and carotenoid pigment which has a potential for application as food supplement and as antioxidant (Aishwarya and Binita, 2018).

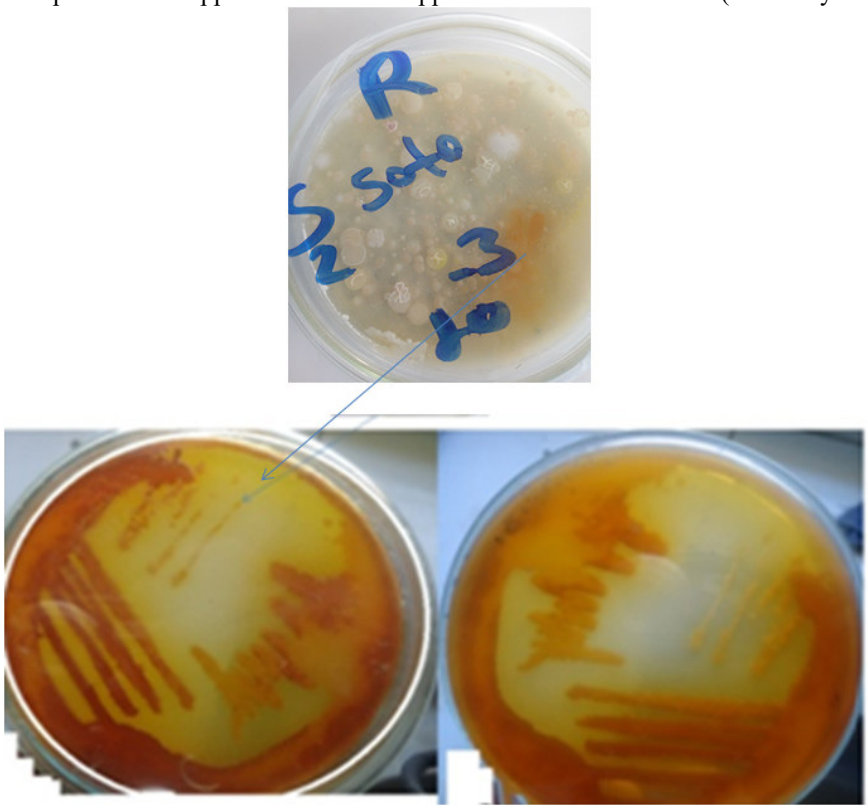

Fig 1. Isolation, purification and intra and extra cellular pigment production by the bacterium isolate Mif41

Morphological and biochemical characterization of the bacterium isolate Mif41

Morphological characterization

Culture characterization of the highly pigmented bacterium isolate was performed by observing and recording size, shape, color, elevation, margin, of the bacterium isolate grown on the surface of agar media (Table 1). Similar reports in the literature indicated that, the growth of bacterial colony on agar media were usually characterized and described in term of form/shape, elevation, margin, appearance, pigmentation (color) and texture and another report, have stated that the green pigment producing bacterial isolates from hot water springs of Bakreshwar, West Bengal were found to be Gram negative with short rods and catalase positive (Mukherjee *et al*., 2012)

Biochemical Characterization

The results of biochemical tests of the yellow pigmented bacterium isolate was presented in (Table 1). The yellow pigmented bacterium isolates was negative for Vogasproskaurs, urease and H2S test while it showed positive for all the rests of the tests. The Gram staining results showed that the bacterium isolate was gram negative and short rod. It has been reported that most of the pigment producing bacterial were Gram negative with bacilli or rod shape. (Mukherjee *et al*., 2012).

Table 1.Morphological and biochemical characteristics of the bacterium isolate Mif41

Morphological characteristics		Biochemical characteristics	
Colony Character	Observation	Tests	Observation
Size	Small	Citrate	+
Color	Orange	Indole	+
Shape	circular	Methylred	+
Margin	Entire	vogesproskaurs	-
Elevation	convex	oxidase	+
Opacity	Opaque	catalase	+
Consistency	Sticky	Urease	-
Gram reaction	negative	Triple sugar iron test	+
Cell shape	Small rod	H_2S	-
Motility	Motile		
O/F utilization of sugar	Oxidative		
Suggested genus	*Pseudomonas* sp		

Factor affecting pigment production by the bacterium isolate Mif41

Effects of carbon sources on growth and pigmentation of the bacterium isolate Mif41

The effect of different carbon sources on the growth and pigmentation of the bacterium isolate was presented by (Fig 2 and Table 2) which indicated that the bacterium isolate was able to grow on all the examined carbon sources. However, no pigment production was observed for both sorbitol and glucose. Similar work was reported by Ramendra *et al*. (2016) who stated that seven carbon sources (starch, fructose, maltose, glucose, lactose, xylose and sucrose) enhanced pigment production. Different nutrients have their own effect on growth of the bacterial isolates. It is known that, to improve the efficiency of pigment production, it is important to optimize the effects of various physiological conditions such as carbon and nitrogen sources (Ramendra *et al.,* 2016). There was significant ($p \leq 0.001$) difference between the means for pigment production for both extracellular and intracellular pigments at 28^0C for 48hrs by the bacterium isolate Mif41 when it was analyzed by optical density. The highest pigment production intensity was observed with fructose (1.10 g/50 ml) followed by lactose and sucrose for both intracellular and extracellular pigments. Other carbon sources like dextrose, myo-oinsotol, starch, mannitol and maltose were also favored the growth but pigment intensity was less when compared with fructose and lactose (*Table 2*.).

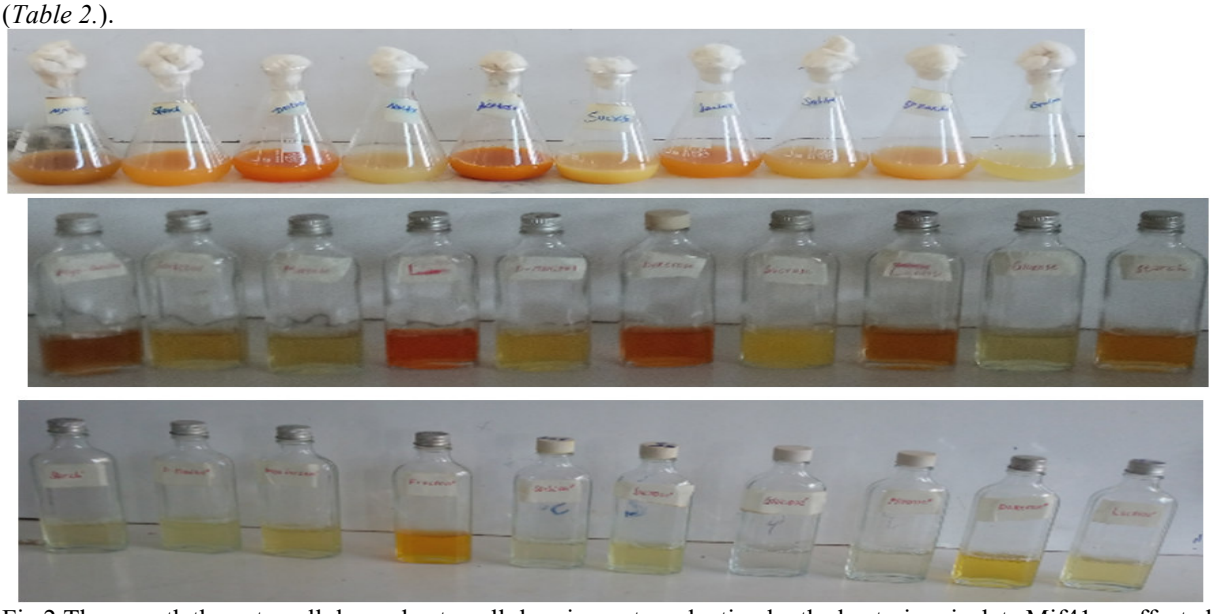

Fig 2.The growth the extracellular and extracellular pigment production by the bacterium isolate Mif41 as affected by the nature of carbon sources.

Table 2..Effect of Carbone Source on production of extracellular and intracellular pigments.

Carbone source	OD of extracellular pigments	OD intracellular pigments
Fructose	1.10	0.70
Lactose	0.90	0.60
Dextrose	0.80	0.50
Myo-insotol	0.70	0.40
Starch	0.65	0.35
Sucrose	0.50	0.30
Mannitol	0.40	0.20
Maltose	0.30	0.25
Sorbitol	0.25	0.17

Effect of Nitrogen Sources on growth and pigmentation of the bacterium isolate Mif41

The effect of nitrogen sources on the growth and pigmentation of the bacterium isolate was shown in Table 3. The organic sources of nitrogen favored more growth and pigmentation, while the inorganic sources of nitrogen were found to be inhibitory for both growth and pigmentation. There was significant ($p \leq 0.001$) difference between the means of production of extracellular and intracellular pigments at 28^0C for 48hr. Among the nitrogen sources tested, yeast extract gave a maximum optical density at wave length of 515 nm for both extracellular (1.20 g/xx) and intracellular (0.80 g/xx). On the other hand, nitrogen sources like peptone, milk powder and treptone are also favored the growth but pigment intensity was less when compared with yeast extract. Weakest grow and pigmentation was observed on tryptone (0.40). Therefore, the bacterium isolate produce optimum pigments when medium supplemented with yeast extract and peptone which were the best nitrogen source according to this finding. Generally, the chosen nitrogen sources like yeast extract, peptone, milk powder and treptone did exert a strong influence on the pigment production. However, minimum optical density values were observed at wavelength 515 nm (0.00) for both ammonium sulphate and potassium nitrate for both intracellular and extracellular pigmentation. This finding was in line with results reported by (Ramendra *et al*., 2016) that pointed out that, the chosen nitrogen sources like yeast extract, peptone, Milk powder and tryptone did exert a strong influence on the pigment production (Cecilia *et al*., 2020) indicated that from five nitrogen sources, peptone was found to be the best substrate for cellular growth and pigment production in both isolates.

Table 3.Effect of Nitrogen source on Specific production of extracellular and intracellular pigments

Nitrogen source	OD of extracellular pigments	OD intracellular pigments
Yeast extract	1.20	0.80
peptone	0.80	0.60
Milk	0.60	0.40
Potassium nitrate	0.00	0.00
Ammonium sulphate	0.00	0.00

Effect of pH on growth and pigment production by bacterium isolate Mif41

The medium pH variously influenced the growth and pigmentation of the bacterium isolate and maximum growth and pigmentation were observed near the neutral pH, while the more acidic and more basic PH of the medium showed inhibitory effects on both the growth and pigmentation (Table 4). Both intracellular and extracellular pigment was maximum at pH 8 with maximum absorption of 1.2. The result indicated that the bacterium isolate was able to grow at wide range of pH values (4.5 – 10.0) with optimum growth between 6.0 and 10.0 and the minimum growth at pH 4.5 (acidity) for extracellular and pH 6 (near neutral) for intracellular. It is known that the slight change in pH may change the shade color of microbial pigment and it varies from one microorganism to another (Joshi *et al*.,2003; Ramendra *et al.,* 2016) and the study conducted by Hizbullahi *et al*. (2018) on the production and characterization of orange pigment produced by halophilic bacterium isolated from abattoir soil where indicated higher pigment produced near pH 7 but gradually decline toward alkaline and the lowest production of pigments was observe at pH 2. In pigment production from bacterial isolates, optimization of the pH is very important work because it is one of the physiological factors for microbial pigment production (Joshi *et al*., 2003).

Table 4.Specific production of extracellular and intracellular pigments

PH tested	OD of extracellular pigments	OD intracellular pigments
4.5	0.60	0.40
6	0.70	0.50
7	0.80	0.60
8	1.00	0.80
10	0.80	0.60

Effect of incubation Temperature on pigment production by bacterium isolate Mif41

Highly significant differences were observed for both the intracellular and extracellular pigments by the spectrophotometric analysis ($p \leq 0.001$) at different temperature incubation. The optimum temperatures for growth and pigment production of both extra- and intracellular pigments took place between 20-28^{0}C (Fig3 and Table5). In this study, pigment was produced between 20^{0}C and 45^{0}C with maximum production at 20^{0}C (Fig 3) with OD value of 1.1 at 515nm. The results of this is similar with the results reported by (Usman *et al*., 2018), who indicated that, the UV-Visible results of the extracted pigments was generated at a wavelength region between 200-800nm. The orange pigment produced by *Salinococcus roseus* showed highest peak of 450nm which gradually declined toward visible region. The highest absorption of orange pigment at 450nm might be attributed to conjugated bonds of the pigment. This implies that the highest absorption at 450nm is an indication that the orange pigment belong to carotenoid family. In the previous study, the temperature increment from 4^{0}C to 25^{0}C favoured the production of yellow pigment but dramatically decreased beyond 25^{0}Cto 37^{0}C (Aishwarya and Binita, 2018). Similarly, the identification and characterization of extracellular red pigment producing bacteria isolated from soil showed maximum production of pigment between 30-37^{0}C (Kumar *et al*., 2015) and the authors further commented that production of microbial pigments is determined by the type of microorganism and incubation temperature. The growth and production of pigments began to decline steadily as temperature increased far beyond optimum value. In another study, 45^{0}C was the optimum temperature for the growth of bacteria (Goswami *et al*., 2010).It is known that temperature is the main factor that determines production of bacterial pigments ((Kumar *et al*., 2015).

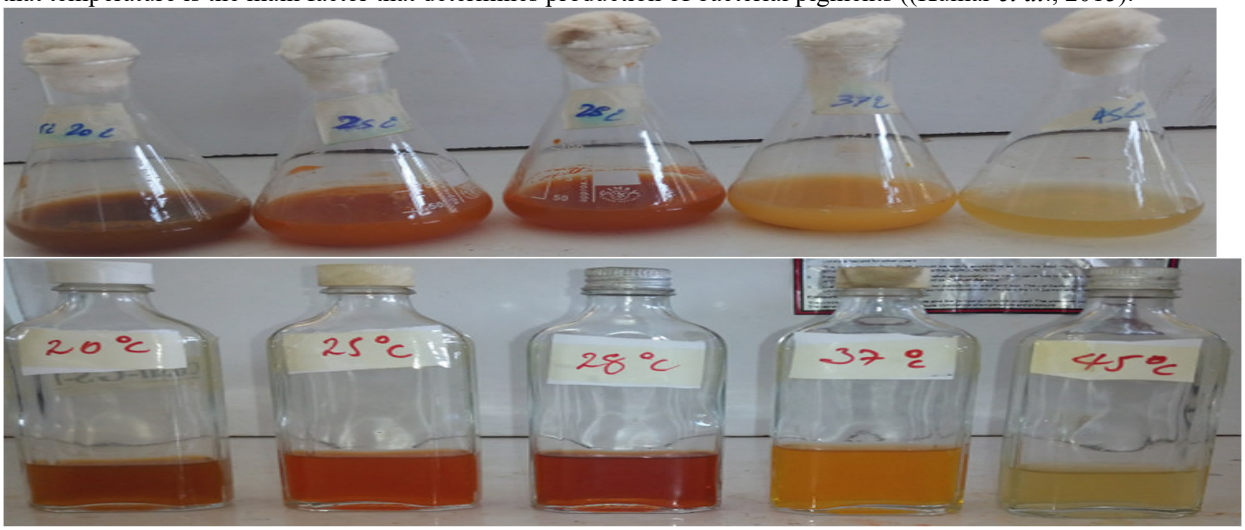

Fig3..The growth and pigmentation of the bacterium isolate Mif41 as affected by incubation temperature

Table 5..Effect of temperature on the specific production of extracellular and intracellular pigments.

Temperature	OD of extracellular pigments	OD intracellular pigments
20ºc	1.10	0.90
25ºc	0.90	0.800
28ºc	0.80	0.70
37ºc	0.70	0.55
45ºc	0.50	0.50

Effect of Different Concentration of Salts (NaCl) on growth and pigmentation of the bacterium isolate Mif41

The pigment production ability of the bacterium isolate at different concentration of salt was presented in Table 6**.** Maximum pigment production was observed at salt concentration of 0.5 % for both intracellular and intracellular pigments. The production of pigment for extracellular was decreased dramatically as salt concentration in the medium increased from 0.5% to 5%. This result was in line with the reports of (Aishwary and Binita, 2018), who stated that, production of extracellular pigment was enhanced at 0.5% concentration. Highly significant differences were observed for both the intracellular and extracellular pigments by the spectrophotometric analysis ($p \leq 0.001$) due to the differences in concentration of salts in the medium. In this study, the maximum pigment was produced at salt concentration of the 0.5% (OD.0.65 and 0.45 respectively. Reports in the literature showed that, on optimizing the conditions for pigment production by bacterial strain *Halorubrumsodomense* isolated from water samples of solar salt lake which indicate the maximum pigment production with 30 per cent NaCl concentration (Khanafari *et al.,* 2010)., indicating different microorganisms have different ability to grow and produce products in different concentration of slats.

Table 6. Effect of different concentration of salt on pigment production of the bacterium isolate as indicated by OD

Salt concentration	OD of extracellular pigments	OD intracellular pigments
0.5%	0.65	0.45
1%	0.54	0.20
2%	0.51	0.40
3%	0.50	0.35
4%	0.20	0.15
5%	0.00	0.00

Conclusions

The results of this study indicated that there was huge diversity of pigmented bacteria in the rhizosphere of trees. The bacterium isolate Mif41 was characterized from morphological and biochemical point of view and tentatively identified as the species of *Pseudomonas*. The optimization of nutritional and physiological conditions for growth and pigmentation showed that, the bacterium isolate able to produce different colored compounds under various conditions. Finally, ability of the bacterium isolate Mif41 to produce different colored pigments under different nutritional and environmental conditions suggested that this bacterium isolate will be considered as a candidate for the industrial production of different pigments which will be applied in various industries

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